

CLAIMS

What is claimed is:

1. An isolated polynucleotide comprising a first nucleotide sequence encoding a polypeptide of at least 50 amino acids that has at least 60% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a plant lecithin:cholesterol acyltransferases polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:14, or a second nucleotide sequence comprising the complement of the first nucleotide sequence.
2. The isolated polynucleotide of Claim 1, wherein the first nucleotide sequence consists of a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, and 13 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, and 14.
3. The isolated polynucleotide of Claim 1 wherein the nucleotide sequences are DNA.
4. The isolated polynucleotide of Claim 1 wherein the nucleotide sequences are RNA.
5. A chimeric gene comprising the isolated polynucleotide of Claim 1 operably linked to suitable regulatory sequences.
6. An isolated host cell comprising the chimeric gene of Claim 5.
7. An isolated host cell comprising an isolated polynucleotide of Claim 1 or Claim 3.
8. The isolated host cell of Claim 7 wherein the isolated host is selected from the group consisting of yeast, bacteria, plant, and virus.
9. A virus comprising the isolated polynucleotide of Claim 1.
10. A polypeptide of at least 50 amino acids that has at least 60% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of a plant lecithin:cholesterol acyltransferases polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:14.
11. A method of selecting an isolated polynucleotide that affects the level of expression of a plant lecithin:cholesterol acyltransferases polypeptide in a plant cell, the method comprising the steps of:
  - (a) constructing an isolated polynucleotide comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13 and the complement of such nucleotide sequences;
  - (b) introducing the isolated polynucleotide into a plant cell;

(c) measuring the level of a polypeptide in the plant cell containing the polynucleotide; and

(d) determining the level of polypeptide in the plant cell to provide a positive selection means.

5 12. The method of Claim 11 wherein the isolated polynucleotide consists of a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, and 13 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, and 14.

10 13. A method of selecting an isolated polynucleotide that affects the level of expression of a plant lecithin:cholesterol acyltransferases polypeptide in a plant cell, the method comprising the steps of:

(a) constructing an isolated polynucleotide of Claim 1;  
(b) introducing the isolated polynucleotide into a plant cell;  
(c) measuring the level of polypeptide in the plant cell containing the  
15 polynucleotide; and  
(d) determining the level of polypeptide in the plant cell to provide a positive selection means.

14. A method of obtaining a nucleic acid fragment encoding a plant lecithin:cholesterol acyltransferases polypeptide comprising the steps of:

20 (a) synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13 and the complement of such nucleotide sequences; and

(b) amplifying a nucleic acid sequence using the oligonucleotide primer.

25 15. A method of obtaining a nucleic acid fragment encoding a plant lecithin:cholesterol acyltransferases polypeptide comprising the steps of:

(a) probing a cDNA or genomic library with an isolated polynucleotide comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13  
30 and the complement of such nucleotide sequences;

(b) identifying a DNA clone that hybridizes with the isolated polynucleotide;

(c) isolating the identified DNA clone; and

(d) sequencing the cDNA or genomic fragment that comprises the isolated DNA clone.

(a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a plant lecithin:cholesterol acyltransferases, operably linked to suitable regulatory sequences;

5 (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the plant lecithin:cholesterol acyltransferases encoded by the operably linked nucleic acid fragment in the transformed host cell;

(c) optionally purifying the plant lecithin:cholesterol acyltransferases expressed by the transformed host cell;

10 (d) treating the plant lecithin:cholesterol acyltransferases with a compound to be tested; and

(e) determining the activity of the plant lecithin:cholesterol acyltransferases that has been treated with a test compound thereby selecting compounds with potential for inhibitory activity.

15 17. An isolated polynucleotide comprising at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, and the complement of such sequences.

18. A method of positive selection comprising:

(a) transforming a plant cell with a chimeric gene of Claim 5; and

20 (b) growing the plant cell to allow expression of the polynucleotide to alter the concentration of phytosterol in the cell to provide a positive selection means.